

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of ) **MAIL STOP AMENDMENT**  
)  
Bo Runeman et al. ) Group Art Unit: 1796  
)  
Application No.: 10/689,762 ) Examiner: OGDEN JR, NECHOLUS  
)  
Filed: October 22, 2003 ) Confirmation No.: 6021  
)  
For: HYGIENE TISSUE WITH LACTIC )  
ACID PRODUCING BACTERIAL )  
STRAINS )

DECLARATION UNDER 37 C.F.R. § 1.132 OF EVA GRAHN HÅKANSSON

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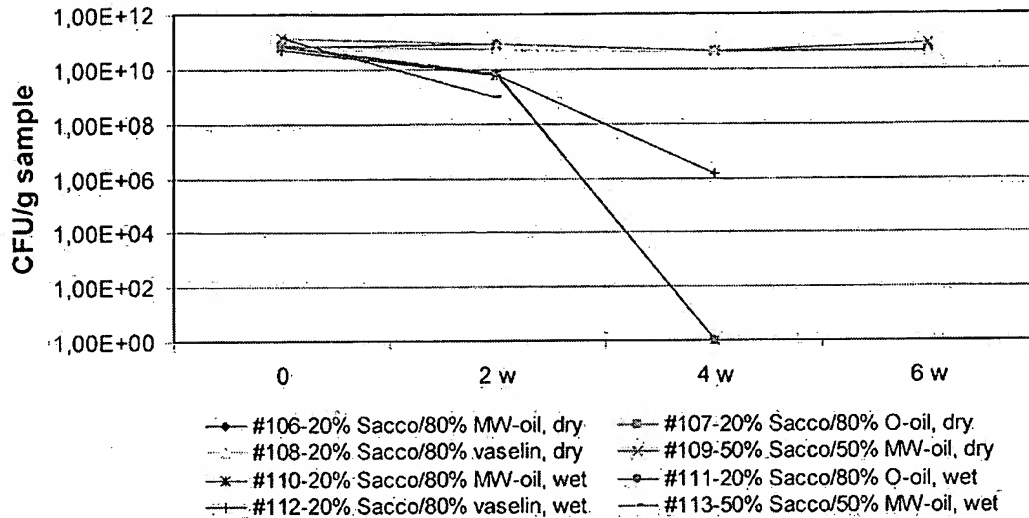
Sir:

I, Eva Grahn Håkansson, hereby declare and state the following:

1. I am an employee of Essum Probiotics AB. I have a PhD, Dr.Med.Sc. in Clinical Bacteriology and have 30 years of experience in the area of clinical bacteriology. I am also a qualified person for the production of probiotic lactobacilli at Probac AB and have experience studying survival of different lactobacillus products.
2. I have read and reviewed Gordon (USPN 5,763,332) and Farmer (WO 01/13956).
3. **Water Activity** - Water activity or  $a_w$  is a measurement of the energy status of the water in a system. It is defined as the vapor pressure of water divided by that of pure water at the same temperature; therefore, pure distilled water has a water activity of exactly one. Water activity better predicts the growth of microorganisms because microorganisms can only use "available" water. Thus, the water activity is not inherent to LAB, but is a feature of a preparation as a whole.

4. Experiments have been conducted to show the effect of increased water activity on the survival rate of LAB. A *Lactobacillus plantarum* (LB 931) was produced, concentrated, freeze dried and milled to a dry powder ( $a_w = 0.182$ ). Sucrose was used as protective sugar and dry corn starch was added (50%) to ensure the low and desired water activity. 20-50% of powder was blended into petrolatum or paraffin oils according to the figure above. (MW-oil, and O-oil are two paraffin oils, Vaseline is petrolatum).
5. The blends were put into wells of stainless steel. (3 mm deep and 17 mm in diameter). The samples were stored in 37 °C either in a dry chamber (refers to dry in the figure below) or in an chamber with a tray with water in the bottom (results in almost 100% rH in the atmosphere) (refers to wet in the figure below). Sample weights were 0,117g. The number of surviving Lactobacillus was measured every second week according to the figure using spread plate technique and MRS agar.
6. The results show that for dry Lactobacillus in oil or petrolatum stored in a dry atmosphere (sample106-109) – the storage stability is very good. The results further show that for Lactobacillus in oil or petrolatum stored in high humidity (sample 110-113) – the humidity has reached the Lactobacillus granules – increased water activity and hereby also dramatically decreased the storage stability.

**Survival of LB931 freeze dried powder in oils and petrolatum. Stored at 37 C in high or low humidity.**



7. **Alcohol/Glycol** - Alcohols, ethanol for example, are known to be effective antimicrobials. See also *McDonnell G, Russell AD (1999)*, "Antiseptics and disinfectants: activity, action, and resistance". Clin. Microbiol. Rev. 12 (1): 147-79. PMID 9880479. Alcohols exhibit rapid broad spectrum antimicrobial activity. See also *McDonnell G, Russell AD (1999)*. Glycols, such as propylene glycol, hexylene glycol and 1,3-butylene glycol, are known to have antimicrobial properties. See also *Kinnunen T, Koskela M, Acta Derm Venereol, 1991; 71(2):148-50*, Antibacterial and antifungal properties of propylene glycol, hexylene glycol, and 1,3-butylene glycol in vitro. Additionally, polyethylene glycol has been shown to have significant antibacterial activity. See also *J Chirife et al., Antimicrob Agents Chemother. 1983 September; 24(3): 409-412*, In vitro antibacterial activity of concentrated polyethylene glycol 400 solutions.

8. I have reviewed Example VI of Gordon. Considering Example VI of Gordon, please note that, generally, Lactobacillus (LB) that is freeze-dried has a long shelf life if it is kept at room temperature and away from humidity, oxygen and heat.

9. Example VI of Gordon presents an absorbent article with a high internal phase emulsion. Of the high internal phase emulsion, 88.5% of the emulsion is an internal polar phase. The polar phase has (percentage given as percentage of the emulsion):
- Sodium Carbonate - 0.5%  
Preservative - 0.5%  
Denatured Ethanol - 40%  
Distilled Water - 47.5%
10. With the water content of 47.5%, the lactobacillus will absolutely have a very short survival (just some days) in room temperature as the water activity will be far above 0.3  $a_w$ . There is also 40% ethanol that is, as anyone skilled in the art of working with microorganisms would know, very harmful for the survival of lactobacillus or any other bacteria. Ethanol is often used to kill bacteria.
11. Lactobacilli in a freeze-dried powder can survive (in room temperature) if the formulation has a very low water-activity i.e., below 0.3. In example VI in Gordon, there is a very high amount of both water and ethanol and there is nothing in the formulation that is able to bind the water and/or ethanol to reduce the water activity to a low level. Compared to freeze-dried lactobacilli in a lipid phase with a water activity of 0.3  $a_w$  or less, the survival of lactobacilli in example VI is much worse.
12. It is not necessary to do any experiments replicating Example VI of Gordon, since the above remarks are common knowledge to anyone skilled in the art of working with microorganisms.

13. I hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 2008-05-09

By: Eva Grahn Håkansson  
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